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Tube Morphogenesis: No Pipe Dream in *Drosophila*

Dispatch

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Tubular organs have characteristic lumen sizes that are generated during development. Recent studies of tubular systems in *Drosophila* have implicated intracellular vesicle transport as an important step in lumen expansion.

The control of lumen size is important for the normal physiology of tubular organ systems, such as the vasculature, lungs and kidneys. How do these tubes attain their characteristic sizes during development? Work on *Drosophila* has begun to shed light on the mechanism by which tubes form and the pivotal role of the apical membrane in regulating lumen size [1–6].

Tubular epithelia such as the *Drosophila* salivary glands, renal (excretory) tubules and trachea (lungs) are well suited to studies of tube morphogenesis. Genetic screens have identified several mutations that affect morphogenesis of these tissues [1,7,8]. Work on the tracheal system and salivary glands suggests that there are some common genetic and cellular mechanisms that control tube morphology [3,4]. Although they form very different structures, the initial steps in tube formation are common to both organs (Figure 1A,B). A sheet of thickened epithelial cells forms a placode that transforms into a tube through a set of post-mitotic events. First, a group of cells within each placode constrict at the apical surface and are internalized. Next, the sequential invagination of cells leads to the formation of a nascent tube. The tube then elongates and finally undergoes lumen expansion. This last step is critically dependent on apical membrane growth which, together with cell flattening, ensures that the outer tube diameter can remain constant as the lumen expands [1] (Figure 1C). It seems that apical membrane growth occurs, paradoxically, as cells constrict their apical surfaces and invaginate [6] (Figure 1C).

Studies of the tracheal system and salivary glands have identified transcription factors that affect apical membrane development. One is the product of the *ribbon* (*rib*) gene, a nuclear protein required for the proper morphogenesis of the tracheal system, salivary glands and other tissues [1–4]. *Rib* mutant embryos form stunted tracheal tubes [3,4]. The basal surfaces of *rib* mutant tracheal cells can respond to cues that control their outgrowth, but their apical membranes fail to follow [4]. The defect in *rib* mutants may be due to the lack of apical membrane growth and/or an inability to couple basal outgrowth with that of the apical surface [4]. Hemphälä *et al.* [5] have identified another transcription factor, product of the *grainy head* (*grh*) gene, which normally restricts apical membrane growth in the tracheal system: *grh* mutants develop excessive apical membranes and form abnormally elongated tubes. Overexpression of *grh* results in severely reduced apical membrane, and the trachea form narrow lumens (Figure 1C). The cellular mechanism by which Grh regulates membrane development is not known, but Hemphälä *et al.* [5] speculate that it may influence apically directed membrane vesicle trafficking.

In their recent study of lumen expansion in *Drosophila* salivary glands, Myat and Andrew [6] found that two other transcription factors, products of the *hairy* (*h*) [9] and *huckebein* (*hkb*) genes [10], have opposing effects on apical membrane growth. *H*, like *grh*, normally functions to restrict apical membrane growth: salivary glands of *h* mutant embryos either form branch-like outgrowths or have severely enlarged lumens (Figure 1C). In contrast, *hkb* mutant salivary glands are defective in apical membrane growth and have narrow lumens compared to wild-type, and overexpression of *hkb* causes increased apical membrane growth and results in the formation of dilated salivary glands (Figure 1C).

Genetic epistasis experiments showed that H normally acts as a repressor of *hkb* expression in the salivary glands [6] (Figure 2A). What is the cellular basis by which the transcription factors H and Hkb control lumen expansion? Myat and Andrew [6] have shed light on this question in the course of uncovering the roles of two important players in this process: the *klarsicht* (*klar*) and *crumbs* (*crb*) gene products. Klar [11–13] is a putative regulator of the microtubule motor protein dynein, and Crb is a well known apical determinant [14,15].

Myat and Andrew [6] found that expression of both *klar* and *crb* is elevated in invaginating wild-type salivary gland cells, at the time when increased apical membrane growth takes place. This induction of *klar* and *crb* in invaginating salivary gland cells requires Hkb function. Mutations in *klar* or *crb* produce salivary glands with reduced lumens, and their overexpression results in salivary gland dilation (Figure 1C). Although *crb* and *klar* have similar mutant phenotypes, their wild-type products seem to cause apical membrane growth differently.

Crb is an apical membrane determinant [14,15] required for the biogenesis and maintenance of adherens junctions [16]. Overexpression of *crb* leads to an increase in apical membrane [15]. Recent work on photoreceptor morphogenesis in the *Drosophila* eye has shown that Crb functions in a complex of proteins that includes β_H-spectrin [17,18]. This is especially interesting as it is known that coated pit budding is accompanied by loss of spectrin [19]. This suggests that Crb might increase apical membrane growth by stabilizing the spectrin cytoskeleton and thereby decreasing the rate of endocytosis. While this may be

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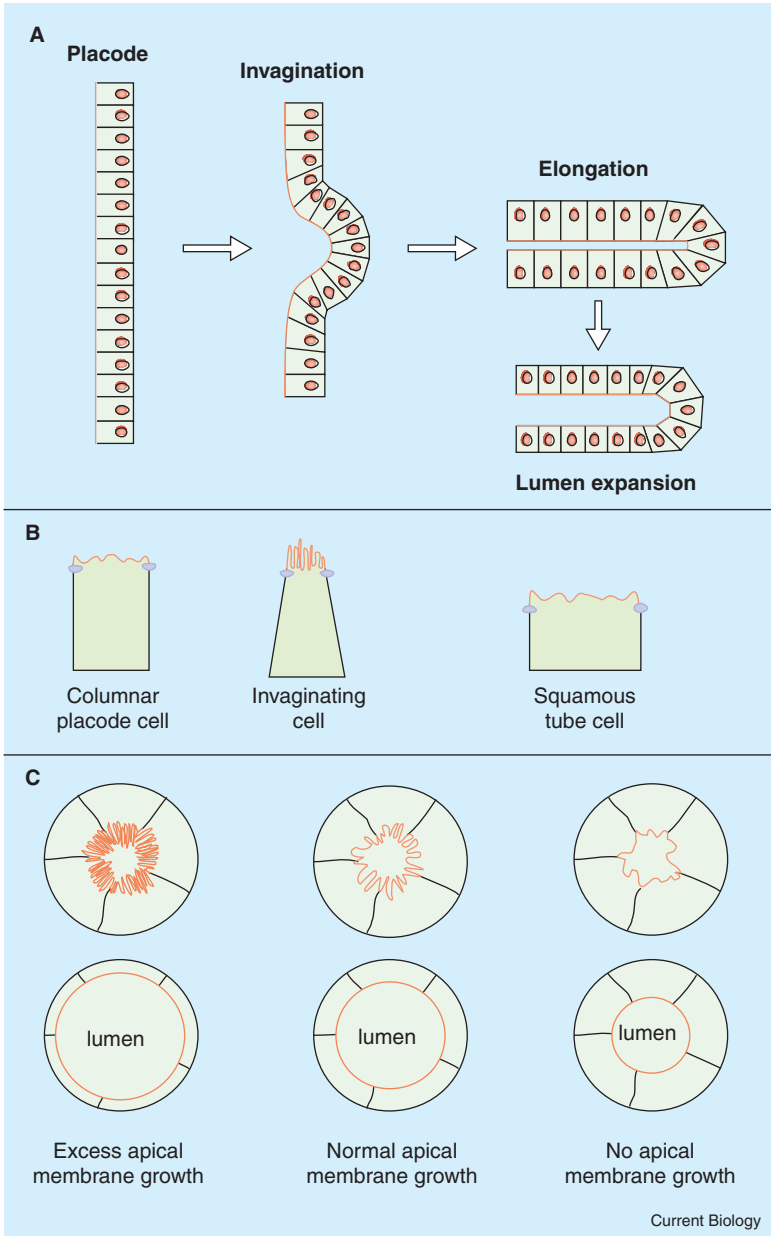

Figure 1. The development of a tubular organ.

(A) Columnar *placode* cells *invaginate* by constricting their apical surfaces (orange). After *invagination* tube cells elongate and then undergo *lumen expansion* by expanding their apical membranes and flattening out. (B) Cell shape changes during tube formation. Cells in the placode are columnar (left) while invaginating cells have narrow, constricted apical surfaces (middle). At this stage, increased growth of apical membrane is seen. After lumen expansion has taken place, tube cells are flat and squamous (right). Zonula adherens are indicated as mauve ovals. (C) The effect of apical membrane growth on lumen diameter. Excessive apical membrane growth (left) results in abnormally large lumens compared to wild-type (center). Reduced or no apical membrane growth results in tubes with narrow lumens (right).

the case in the salivary glands, new findings by Myat and Andrew [6] indicate that there may also be post-transcriptional regulation of *crb*. When ubiquitously expressed, *crb* mRNA is detected throughout the salivary placode but the protein is detected only in a subset of cells, those that invaginate. But co-expression of *crb* with either *hkb* or *klar* leads to increased levels of Crb throughout the salivary placode. This suggests that the post-transcriptional stabilization of Crb requires Hkb (presumably acting via *klar*, see below).

Myat and Andrew [6] propose a different, tantalizing model for the function of Klar. As Klar is thought likely to be a regulator of dynein, they suggest that it promotes the transport of membrane vesicles to the apical surface, thereby causing polarized membrane growth (Figure 2B). Consistent with this idea, salivary gland cell microtubules are oriented with their minus ends toward the apical surface. An antibody that recognizes the apical surface of the salivary gland also reveals punctate vesicle-like staining inside the cells. Overexpression of *hkb* leads to expansion of the lumen and a reduction in the vesicle-like staining. In contrast, electron micrographs of *hkb* mutants reveal reduced apical membranes and an accumulation of electron-dense vesicles in the salivary gland cells. On the basis of genetic epistasis data, Myat and Andrew [6] suggest that the altered distribution of vesicles seen when *hkb* is manipulated is caused, indirectly, by its effects on *klar* expression. It must also be noted that H can repress *klar* expression independently of Hkb, revealing a further level of regulation (Figure 2A).

The data of Myat and Andrew [6] can be summarized in the following model (Figure 2A,B). The transcription factor H represses the expression of *hkb* and

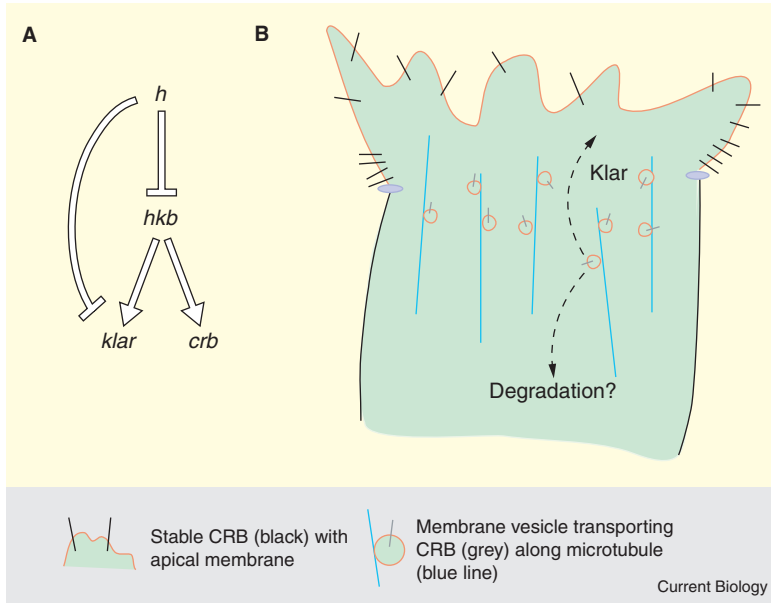

Figure 2. Genetic analysis of tube formation in *Drosophila*.

(A) Genetic hierarchy during salivary gland morphogenesis. *H* represses *hkb* and *klar* expression, while *hkb* promotes *klar* and *crb* expression. (B) The model proposed by Myat and Andrew [6]. According to this model, an invaginating cell increases apical membrane as a result of Hkb-induced expression of *klar* and *crb*. Klar promotes vesicle traffic to the apical surface in a microtubule (blue lines)-dependent manner. This leads to apical membrane growth and stabilization of Crb at the apical surface, which also contributes to membrane growth.

klar. Hkb in turn promotes the expression of *crb* and *klar*. Crb specifies and confers apical membrane character, while Klar increases membrane growth by promoting apical vesicle traffic. Although this model is attractive, it would be even more compelling if it can be shown that changes in vesicle-like structures caused by various genetic manipulations have a direct, causal relationship with apical membrane growth and Crb stability.

Nevertheless, the new findings may be generally applicable, as preliminary experiments suggest that Klar also increases Crb accumulation in the tracheal system (M.M. Myat and D.J. Andrew, personal communication). This raises the question of whether Rib and Grh have roles to play in regulating vesicle transport. The new studies described here provide a framework for further research in tube morphogenesis. While our understanding of polarized membrane traffic in cultured vertebrate cells has increased over the past few years [20], combining cell biological and genetic approaches will only continue to provide wonderful insights into how organs develop and function *in vivo*.

References

1. Beitel, G.J. and Krasnow, M.A. (2000). Genetic control of epithelial tube size in the *Drosophila* tracheal system. Development *127*, 3271–3282.
2. Jack, J. and Myette, G. (1997). The genes *raw* and *ribbon* are required for proper shape of tubular epithelial tissues in *Drosophila.* Genetics *147*, 243–253.
3. Bradley, P.L. and Andrew, D.J. (2001). *ribbon* encodes a novel BTB/POZ protein required for directed cell migration in *Drosophila melanogaster.* Development *128*, 3001–3015.
4. Shim, K., Blake, K.J., Jack, J. and Krasnow, M.A. (2001). The *Drosophila ribbon* gene encodes a nuclear BTB domain protein that promotesepithelial migration and morphogenesis. Development *128*, 4923–4933.
5. Hemphälä, J., Uv, A., Cantera, R., Bray, S. and Samakovlis, C. (2003). *Grainy head* controls apical membrane growth and tube elongation in response to Branchless/FGF signalling. Development *130*, 249–258.
6. Myat, M.M. and Andrew, D.J. (2002). Epithelial tube morphology is determined by the polarized growth and delivery of apical membrane. Cell *111*, 879–891.
7. Jack, J. and Myette, G. (1999). Mutations that alter the morphology of the malpighian tubules in *Drosophila.* Dev. Genes Evol. *209*, 546–554.
8. Liu, X., Kiss, I. and Lengyel, J.A. (1999). Identification of genes controlling malpighian tubule and other epithelial morphogenesis in *Drosophila melanogaster.* Genetics *151*, 685–695.
9. Howard, K. and Ingham, P. (1986). Regulatory interactions between the segmentation genes *fushi tarazu*, *hairy*, and *engrailed* in the *Drosophila* blastoderm. Cell *44*, 949–957.
10. Weigel, D., Jurgens, G., Klingler, M. and Jäckle, H. (1990). Two gap genes mediate maternal terminal pattern information in *Drosophila.* Science *248*, 495–498.
11. Welte, M.A., Gross, S.P., Postner, M., Block, S.M. and Wieschaus, E.F. (1998). Developmental regulation of vesicle transport in *Drosophila* embryos: forces and kinetics. Cell *92*, 547–557.
12. Fischer-Vize, J.A. and Mosley, K.L. (1994). *Marbles* mutants: uncoupling cell determination and nuclear migration in the developing *Drosophila* eye. Development *120*, 2609–2618.
13. Mosley-Bishop, K.L., Li, Q., Patterson, L. and Fischer, J.A. (1999). Molecular analysis of the *klarsicht* gene and its role in nuclear migration within differentiating cells of the Drosophila eye. Curr. Biol. *9*, 1211–1220.
14. Tepass, U., Theres, C. and Knust, E. (1990). *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. Cell *61*, 787–799.
15. Wodarz, A., Hinz, U., Engelbert, M. and Knust, E. (1995). Expression of *crumbs* confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila.* Cell *82*, 67–76.
16. Grawe, F., Wodarz, A., Lee, B., Knust, E. and Skaer, H. (1996). The *Drosophila* genes *crumbs* and *stardust* are involved in the biogenesis of adherens junctions. Development *122*, 951–959.
17. Pellikka, M., Tanentzapf, G., Pinto, M., Smith, C., McGlade, C.J., Ready, D.F. and Tepass, U. (2002). Crumbs, the *Drosophila* homologue of human Crb1/RP12, is essential for photoreceptor morphogenesis. Nature *416*, 143–149.
18. Izadoost, S., Nam, S.C., Bhat, M.A., Bellen, H.J. and Choi, K.W. (2002). *Drosophila* Crumbs is a positional cue in photoreceptor adherens junctions and rhabdomeres. Nature *416*, 178–183.
19. Kamal, A., Ying, Y. and Anderson, R.G. (1998). Annexin VI-mediated loss of spectrin during coated pit budding is coupled to delivery of LDL to lysosomes. J. Cell Biol. *142*, 937–947.
20. Mostov, K.E., Verges, M. and Altschuler, Y. (2000). Membrane traffic in polarized epithelial cells. Curr. Opin. Cell Biol. *12*, 483–490.